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Structural Studies of MoeA Point Mutants

S. Xiang (SUNY, Stony Brook), J. Nichols (Duke U. Medical Center), K. Rajagopalan (Duke U. Medical Center) and H. Schindelin (SUNY, Stony Brook)

Beamline(s): X26C

Introduction: Molybdenum cofactor (Moco) biosynthesis is an evolutionarily conserved pathway present in archaea, eubacteria and eukaryotes. In humans, genetic abnormalities in the biosynthetic pathway result in Moco deficiency, which is accompanied by severe neurological symptoms and death shortly after birth. The *Escherichia coli* MoeA and MogA proteins are involved in the final step of Moco biosynthesis, the incorporation of molybdenum into molybdopterin (MPT), the organic pyranopterin moiety of Moco. The crystal structure of *E. coli* MoeA has been solved at 2 Å resolution and reveals that the highly elongated MoeA monomer consists of four clearly separated domains, one of which is structurally related to MogA indicating a divergent evolutionary relationship between both proteins. The active form of MoeA is a dimer, and a putative active site appears to be localized to a cleft formed between domain II of the first monomer and domains III and IV of the second monomer **[1]**. Based on the structure, several inactive MoeA mutants have been designed and their activity tested. The parallel structural studies of these mutants provide additional information as to the mechanism of the reaction.

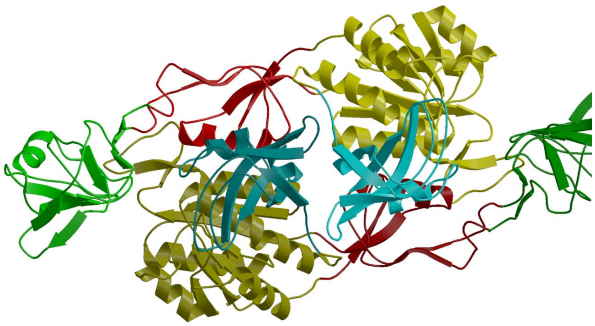

Figure 1. Structure of the MoeA dimmer viewed along the twofold axis Corresponding domains are shown in different shades of the same color: domain I in red, domain II in green, domain III in yellow and domain IV in blue.

Methods and Materials: Seven MoeA point mutant structures (D59N, T100W, T100A, R137Q, D142N, E188A, D228A) have been solved by molecular replacement method, at resolutions between 2 Å and 3 Å. With the exception of R137Q crystals, all belong to space group $P2_12_12_1$, and contain one copy of MoeA dimer in the asymmetric unit. R137Q crystals belong to space group $P2_1$ and contain two MoeA dimers in the asymmetric unit. All diffraction data were collected on beam line X26C at the National Synchrotron Light Source at Brookhaven National Laboratory at a wavelength of 1.1 Å on a Quantum IV ADSC CCD detector.

Results: The MoeA monomer is a highly elongated club-shaped molecule and is composed of four clearly independent domains. In the crystal, MoeA is present as a dimer which is formed by interactions involving domains I, III and IV from each monomer. Although the dimmer is also quite elongated, its main chain dimensions are 44 Å by 44 Å by 115 Å, it definitively has a more globular shape than the monomer. The inactivating mutations cause no major changes in the overall structure, the core regions formed by domains I, III and IV are essentially identical to the native structure. Domain II, however, is rather mobile, it undergoes rotational motions around a hinge generated by residues 49 and 153.

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[1] Journal Article: S. Xiang, J. Nicolas, K.V. Rajagopalan and H. Schindelin, “The Crystal Structure of Escherichia coli MoeA and Its Relationship to the Multifunctional Protein Gephrin” Structure, 9, 2001